

Original Research Article

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Effect of Saccharification Methods on Bioethanol Production by Thermophiles from *Eichhornia crassipes*

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ABSTRACT

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Water hyacinth (*Eichhornia crassipes*) represents a promising candidate for fuel ethanol production in tropical countries because of their high availability and high biomass yield. Bioconversion of such biomass to bioethanol could be wisely managed through proper technological approach. In this work, water hyacinth was used as a substrate for bioethanol production by the use of thermophiles. Water hyacinth was pretreated with sulfuric acid (1 M) and sodium hydroxide (1M) for release of free sugars. Acid pretreatment was most effective in comparison to alkali treatment that resulted in formation of 1.73 mg/ml reducing sugar. The sugars after pretreatment produced maximum 4.1 gm/l ethanol by *Bacillus vietnamensis* strain and 3.58gm/l by *Bacillus stearotherophilus*. Thus, the experiment imparts an economic value to water hyacinth that are cleared from choking waterways.

Introduction

Global warming is a direct cause of overuse and continuous exploitation of fossil fuel resources. As a result there is an emerging need to utilize renewable, sustainable, cost effective, ecofriendly and green alternative energy sources (Chum., 2001). Lignocellulosic biomass can be used as an alternative energy producing source because of its abundance, availability and relatively low cost.

Bioethanol being a safe, clean and renewable resource, can be considered as a potential

alternative to fossil fuel (Rezania *et al.*, 2015). However, most of ethanol is mainly produced from either starch or sugar rich crops therefore it may raise land competition between the food production and biomass energy utilization which causes problems in a developing country like India (Das *et al.*, 2015). Thus, waste or unused lignocellulose is considered as more attractive source for bioethanol production (Valentine *et al.*, 2012; Bayrakci and Kocar, 2014). Ethanol also has a higher latent heat of vaporization (855MJ/Kg) as compared to petrol (293MJ/Kg). Ethanol has higher octane number (99) than that of petrol (80-100) (Ganguly *et al.*, 2012) which

makes ethanol a non-polluting and green fuel.

Vast number of waste and non-food lignocellulosic material has been experimented upon for production of ethanol. Water hyacinth or *Eichhornia crassipes* is a aquatic weed which causes major hazard as it grows uncontrolled in water bodies. Water hyacinth, which originated from Amazon basin is known as one of the world's most intractable and invasive weed (Hu. J *et al.*, 2011) and is usually blamed for depleting nutrients and oxygen from water bodies, reducing biodiversity, increasing evapotranspiration leading to destruction of aquatic ecosystem (Malik, 2007; Guerrero-Coronilla *et al.*, 2015). Water hyacinth has a high hemicellulose content (30-55% of dry weight) and can provide hemicellulose sugar for conversion to ethanol. The biomass of water hyacinth has a very low lignin (3.5%) and high amount of hemicellulose (48%) and 18% cellulose content (Nigam, 2002). Also being an aquatic plant it has an advantage of not being in competition to food crops growing on land (Mishima *et al.*, 2008).

Materials and Methods

Microorganisms

Pure Thermophilic bacterial strains *Bacillus vietnamensis* (N2) and *Bacillus stearothermophilus* (N3) were used for conversion of Lignocellulosic waste to ethanol.

Saccharification of water hyacinth plant

Water hyacinth was collected from Yamuna River. The water hyacinth plant was sundried for 3-4 days in sunlight. The dried parts of water hyacinth were powdered in a grinder. The powder was subjected to pretreatment with acid and alkali. 1N Sulphuric acid and 1N NaOH were added to 5 gm each of dried

plant material, respectively and incubated for 24 h at room temperature for hydrolysis to convert lignocellulosic waste into free sugar.

Estimation of sugar

Released sugar was estimated by using dinitrosalicylic acid (DNS) test, 3,5-Dinitrosalicylic acid is an aromatic compound which reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, that absorbs light strongly at 540 nm (Miller, 1959).

Production of bioethanol by fermentation

The fermentation media was prepared by addition of yeast extract (0.2gm), urea (1gm) and dextrose (5gm) into the pretreated Water Hyacinth samples t per 100 ml of fermentation media. 10% thermophilic culture was inoculated in fermentation media. The mouths of the flasks were tightly sealed to maintain microaerobic condition and the fermentation was carried out at 50°C for 7 days and samples were taken from each of the two broths on each day for quantitative estimation of ethanol.

Estimation of ethanol

The concentration of ethanol was estimated using gas chromatography (GC) (Nucon engineer) with a flame ionization detector (FID) and 6 ft. Porapak N packed column (80/100 mesh) using nitrogen gas as the carrier. Flame is ignited at the flame ionization detector port. The injector, detector and oven temperatures were programmed. After reaching the stability, when the oven temperature and detector, injector temperature were at the programmed temperature, a sample is injected from fermentation flask into injector port by using a micro syringe (1 – 10 µl). The oven temperature was held at 80°C. The injector and detector temperature was

maintained at 110°C. External standard was applied for identification and quantification. The peak eluted is noted (using Nuchrome software) and by knowing the area of peak, the concentration of ethanol is calculated using calibration chart and the ethanol was determined in g/l.

Results and Discussion

Water Hyacinth sample was pretreated using Sulphuric acid and Sodium hydroxide. Among acid and alkali, acid was most effective in pretreatment because in acidic pretreatment more amount of sugar was released in comparing to alkali treatment presented in table 1.

The two novel thermophiles which were provided from Codon Biotech Pvt. Ltd was – 1- *Bacillus vietnamensis* (N2) and *Bacillus stearothermophilus* (N3), these cultures were used for bioethanol production.

Estimation of bioethanol

Fermentation was carried out for 7 days and sample was collected every day for estimation of ethanol by gas chromatography.

The Water Hyacinth sample which was treated with Sulphuric acid, produced maximum amount of ethanol i.e., 4.1 g/l by strain N2 *Bacillus vietnamensis* on 4th days (Table 2 and Fig. 1) and 3.58gm/l by strain N3 *Bacillus stearothermophilus* on 5th day (Table 3 and Fig. 2).

The Water Hyacinth sample which was treated with Sodium hydroxide, produced lesser amount of ethanol i.e., 2.12 g/l by strain N3 *Bacillus stearothermophilus* on 2nd day (Table 5 and Fig. 4) and 0.28gm/l by strain N2 *Bacillus vietnamensis* on 3rd day (Table 4 and Fig. 3).

Water Hyacinth is a highly problematic invasive species and it is one of the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolons which eventually form daughter plants. Each plant additionally can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years.

When not controlled, water hyacinth will cover lakes and ponds entirely; this dramatically affects water flow, blocks sunlight from reaching native aquatic plants, and starves the oxygen of water, often killing fish.

In India the most easy and common method of removal of water hyacinth from water bodies is physical extraction of the plants from the water. Thus, these waste plants were selected as substrate for bioethanol production, which could result in production of economically important ethanol from waste plants.

Biofuel production from cellulosic materials greatly depends on the disruption of its complex lignocellulosic structure, which is recalcitrant to biodegradation.

Hence, a suitable and effective pretreatment method should be selected for different cellulosic substrates. Various kinds of pretreatment methods, including acid and alkali were used for cellulose hydrolysis. But in the present study acid gave the highest breakdown and related ethanol production.

According to Qiuzhuo Zhang *et.al* (2016) 1.289 g/L bioethanol was achieved using sulfuric acid pretreated water hyacinth substrate. In another study by Kumari N *et al.*, (2014) fermentation of water hyacinth hydrolysate using pentose fermenting yeast, *Pichia stipitis* yielded an ethanol concentration of 3.193g/L.

Table.1 Concentration of sugar obtained after pretreatment with acid and alkali

S. NO	SAMPLE (1M)	DNS	POTESSIUM SODIUM TATRATE (ml)	DISTILLED WATER (ml)	O.D	CONC. OF SUGAR (mg/ml)
1.	Acid treated Sample 1	2	1	6	1.35	1.73
3.	Acid treated Sample 2	2	1	6	1.33	1.70
4.	Alkali treated Sample 1	2	1	6	0.60	0.77
5.	Alkali treated Sample 2	2	1	6	0.62	0.79

Table.2 Bioethanol production in water hyacinth treated with acid by strain N2 (*Bacillus vietnamensis*)

S.NO	DAYS OF INCUBATION	AMOUNT OF ETHANOL PRODUCED in gm/l
1	1 st	0.65 ±0.01
2	2 nd	1.32 ±.2
3	3 rd	1.39 ±0.32
4	4 th	4.1 ±0.22
5	5 th	1.93 ±0.18
6	6 th	1.62 ±0.22
7	7 th	0.73 ±0.09

Table.3 Bioethanol production in water hyacinth treated with acid by N3 (*Bacillus stearothermophilus*)

S. NO	DAYS	Amount Of Ethanol Produced in gm/l
1	1 st	0.63 ±0.11
2	2 nd	0.83 ±0.15
3	3 rd	3.44 ±0.65
4	4 th	3.24 ±0.48
5	5 th	3.58 ±0.55
6	6 th	1.27 ±0.21
7	7 th	0.23 ±0.25

Table.4 Bioethanol production in water hyacinth treated with alkali by strain N2 (*Bacillus vietnamensis*)

S.NO	DAYS	Amount Of Ethanol Produced in gm/l
1	1 st	0.06 ±0.01
2	2 nd	0.09 ±0.01
3	3 rd	0.28 ±0.011
4	4 th	0.2 ±0.0
5	5 th	0

Table.5 Bioethanol production in water hyacinth treated with alkali by strain N3 (*Bacillus stearothermophilus*)

S.NO	DAYS	Amount Of Ethanol Produced In gm/l
1	1 st	1.55 ±0.14
2	2 nd	2.12 ±0.18
3	3 rd	1.84 ±0.12
4	4 th	0.3 ±0.002
5	5 th	0

Fig.1 Amount of ethanol production vs days graph by N2 (*Bacillus vietnamensis*)

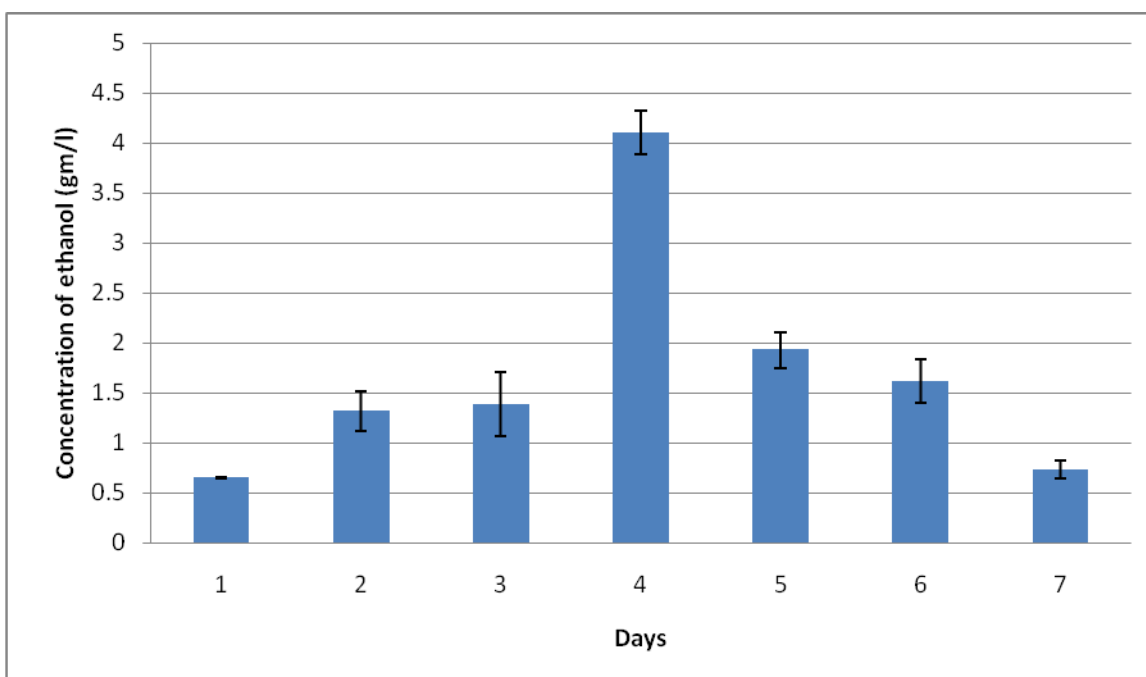


Fig.2 Amount of ethanol production vs days graph by N3 (*Bacillus stearothermophilus*)

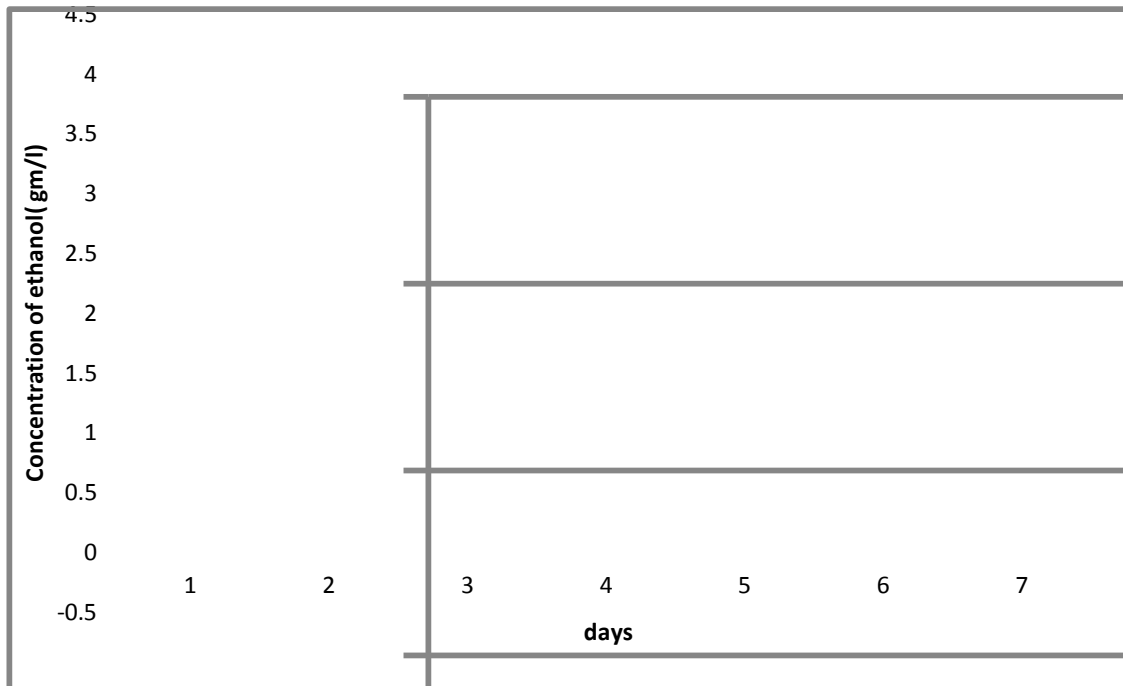


Fig.3 Amount of ethanol production vs days graph by N2 (*Bacillus vietnamensis*)

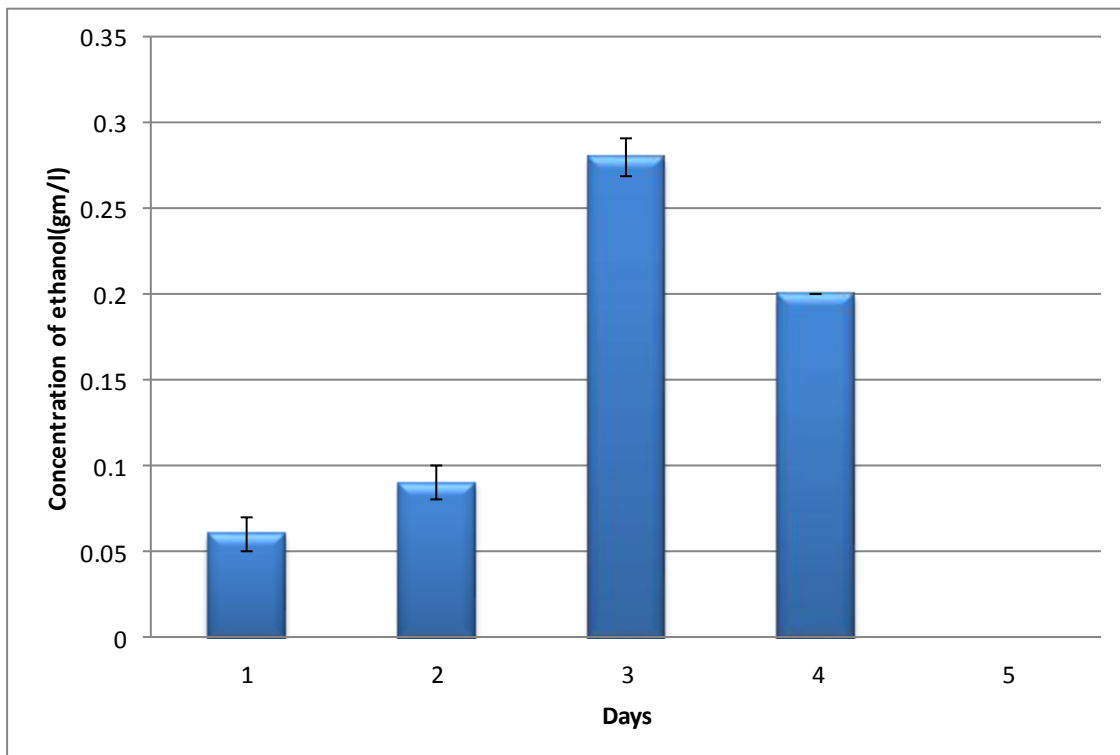


Fig.4 Amount of ethanol produced vs days by N3 (*Bacillus stearothermophilus*)

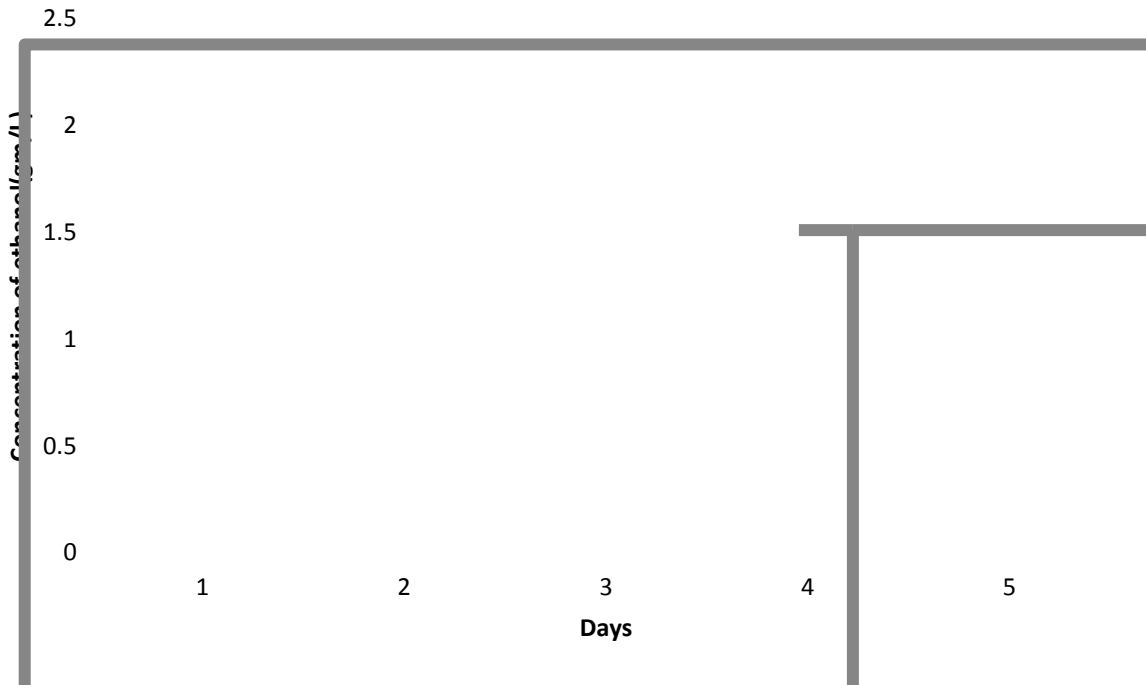
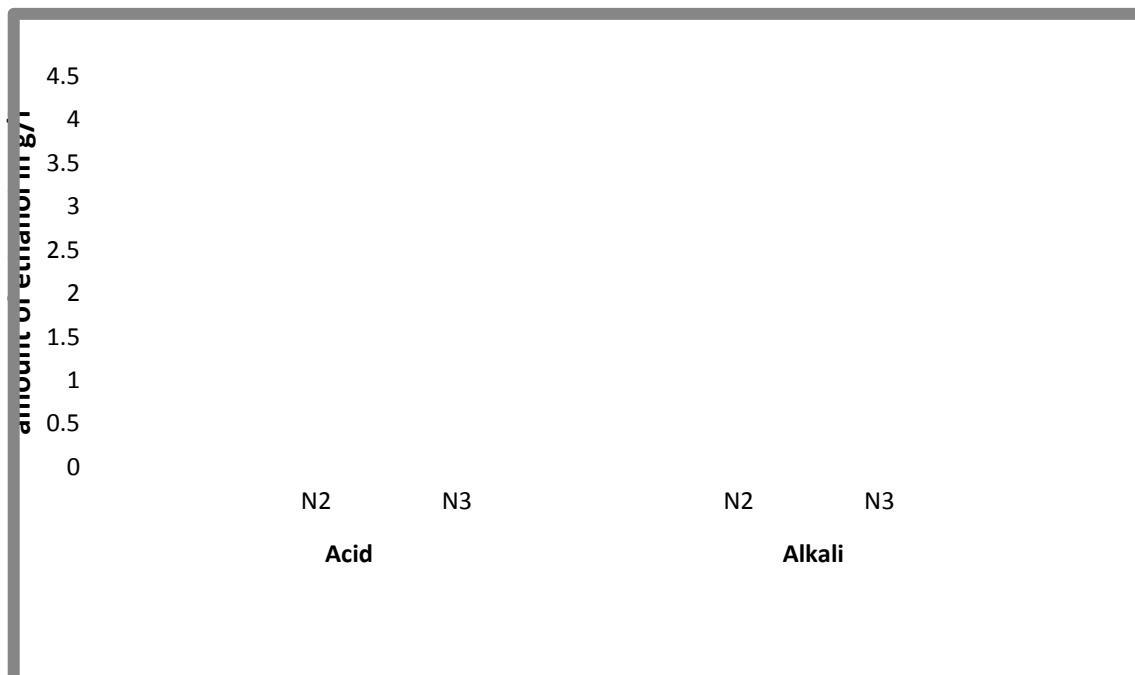


Fig.5 Comparative analysis of ethanol produced by acid and alkali pretreatment



However, in our study acid pretreated water hyacinth substrate produced a maximum of 4.1 gm/l ethanol on 4th day by N2 (*Bacillus vietnamensis*) strain and 3.58 gm/l by N3 (*Bacillus stearothermophilus*) on 5th day.

The alkali pretreated water hyacinth substrate produced a maximum of 0.28gm/l ethanol on 3rd by N2 (*Bacillus vietnamensis*) and 2.12g/l by N3 (*Bacillus stearothermophilus*) on 2nd day.

So acid pretreatment was the most effective method for bioethanol production by water hyacinth compared to alkali pretreatment method.

Water hyacinth (*Eichhornia crassipes*) is one of the world's worst aquatic weeds and it infests rivers, dams, lakes and canals all over the world so it affects the aquatic environment which is a worldwide problem. In the present study we were used this aquatic waste (water hyacinth) as a substrate for bioethanol production to overcome this problem. Bioethanol is one of the most promising replacement for fossil fuel as it is renewable and releases 85% less green-house gases compared to gasoline.

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